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Content of γ-Oryzanol and Composition of Steryl Ferulates in Brown Rice (*Oryza sativa* L.) of European Origin

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The content of γ-oryzanol and the composition of steryl ferulates were determined in brown rice of European origin using on-line coupled liquid chromatography−gas chromatography (LC-GC). Analysis of 30 brown rice samples of various cultivars, grown at different sites and in different seasons, revealed the γ-oryzanol content to range from 26 to 63 mg/100 g. Cycloartenyl ferulate and 24-methylenecycloartanyl ferulate were the major components of γ-oryzanol followed by campesteryl ferulate, campestanyl ferulate, and β-sitosteryl ferulate. The proportions of individual steryl ferulates exhibited enormous variability. However, irrespectively of the great variations observed for single steryl ferulates, the proportions of the sum of 4,4′-dimethylsteryl ferulates (cycloartenyl ferulate, 24-methylenecycloartanyl ferulate) and the sum of 4-desmethylsteryl ferulates (campesteryl ferulate, campestanyl ferulate, and β-sitosteryl ferulate) were rather constant. The significant natural variability observed for γ-oryzanol content and composition of steryl ferulates were shown to be influenced by environmental conditions but not by the degree of maturity of rice grains.

KEYWORDS: γ-Oryzanol; steryl ferulate; brown rice; on-line LC-GC; natural variation; *Oryza sativa*

INTRODUCTION

Ferulic acid esters of phytosterols (steryl ferulates) have been identified in rice, corn, wheat, rye, barley, triticale, Job's tears, and wild rice (*1*−*7*). Among these seeds, rice (*Oryza sativa* L.) exhibits the highest level of steryl ferulates (*7*). The mixture of steryl ferulates found in rice is termed "γ-oryzanol". Ferulic acid esters of 4,4′-dimethylsterols (cycloartenol and 24-methylenecycloartanol) and of 4-desmethylsterols (campesterol, β-sitosterol and campestanol) have been identified as major components of γ-oryzanol (*1*). **Figure 1** shows exemplarily the structure of 24-methylenecycloartanyl ferulate.

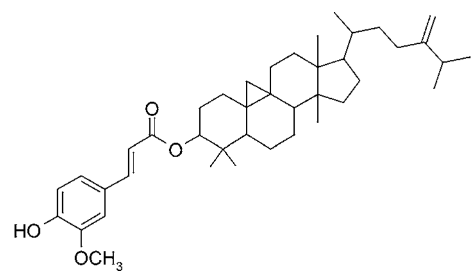

Figure 1. Structure of 24-methylenecycloartanyl ferulate, a major component of γ-oryzanol.

In various in-vitro studies, steryl ferulates exhibited antioxidative activity (*8*−*12*). The addition of γ-oryzanol improved the oxidative stability of vegetable oils at frying temperatures (*13*) and delayed both the development of a rancid off-flavor and the formation of toxic cholesterol oxides in refrigerated cooked beef (*14*). There are indications that the antioxidative activity of γ-oryzanol is influenced by its composition. Oxidation of linoleic acid under UV irradiation was more effectively inhibited by 24-methylenecycloartanyl ferulate and campesteryl ferulate than by cycloartenyl ferulate (*8*). Compared with cycloartenyl ferulate, 24-methylenecycloartanyl ferulate exhibited stronger antioxidative activity against autoxidation of linoleic acid in the dark (*9*). Oxidation of cholesterol was more effectively inhibited by 24-methylenecycloartanyl ferulate than by cycloartenyl ferulate or campesteryl ferulate (*10*).

The most outstanding property of γ-oryzanol is its cholesterol-lowering effect. Oral administration of γ-oryzanol lowered plasma LDL cholesterol in rats and hamsters (*15*−*18*). The cholesterol-lowering activity of γ-oryzanol seems also to be influenced by its composition. Depending on the composition of γ-oryzanol preparations, different effects in hypercholesterolemic rats were observed (*18*). It has been assumed that free 4-desmethylsterols liberated from γ-oryzanol in the gut are responsible for the cholesterol-lowering effect of γ-oryzanol (*19*). In in-vitro studies, pancreatic cholesterol esterase and artificial intestinal juice released β-sitosterol and campesterol from γ-oryzanol but neither cycloartenol nor 24-methylenecycloartanol (*20*). In a similar study, the hydrolysis of sitostanyl ferulate by artificial intestinal juice and pancreatic cholesterol esterase was demonstrated (*21*).

Previous investigations of the natural occurrence of γ-oryzanol focused on the determination of γ-oryzanol contents rather than on the analysis of the composition of steryl ferulates. The effects of genotype and environment as well as kernel thickness or processing on the γ-oryzanol content in rice bran (*22*−*28*) and rice bran oil (*29*−*33*) have been investigated in

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detail. Information on γ-oryzanol content in brown rice is limited. Data reported for γ-oryzanol content in brown rice are based on analysis of one or two individual samples grown in Japan (*34*, *35*), India (*36*), Europe (*37*), China (*37*), or the U.S. (*7*). Due to the limited number of samples analyzed, no conclusions can be drawn regarding the variability in γ-oryzanol content in any of the regions. Despite the fact that γ-oryzanol composition might influence the antioxidative and cholesterol-lowering effect of γ-oryzanol, no data on the variability in composition of steryl ferulates in brown rice is available.

The objective of this study was to investigate variations in both γ-oryzanol content and composition of steryl ferulates in brown rice. Through the use of a rapid and facile methodology based on on-line coupled liquid chromatography−gas chromatography (on-line LC-GC) (*37*), the effects of cultivar, growing location, season, and grain maturity were investigated by analysis of a spectrum of brown rice samples of European origin.

MATERIALS AND METHODS

Samples. One kilogram batches of brown rice and rough rice grains of European cultivars (Cripto, Loto, Selenio, Balilla, Perla, Gladio, Sigalon, Thaibonnet, Ambra, Elio, and Helene) grown organically in Italy, Spain, and France in 2000, 2001, and 2002 were obtained from Mühldorfer Naturkorn-Mühle (Mühldorf, Germany). The material had been dried industrially and exhibited a water content of 13% (personal communication, Kobler, R., Mühldorfer Naturkorn-Mühle, Mühldorf Germany). The grains were stored in the dark at +4 °C and were analyzed within 6 months. Prior to analysis, rough rice grains were manually dehulled by means of a wooden rice dehuller and green grains were sorted out. Green grains of five batches were analyzed in parallel to their mature counterparts.

Determination of γ-Oryzanol Content and Composition of Steryl Ferulates. γ-Oryzanol content and composition of steryl ferulates were determined using a validated on-line LC-GC method described earlier (*37*). About 50 g of brown rice was ground using a cyclone mill, and lipids were extracted from an aliquot of the rice flour (200 mg) with dichloromethane−methanol (2:1, v/v) at 50 °C. The lipid extract was evaporated to dryness by rotary evaporation, and the residue was redissolved in hexane. After passing through a membrane filter, 10 μL of the solution was subjected to on-line LC-GC analysis using a fully automated LC-GC instrument (Dualchrom 3000, ThermoFinnigan, Egelsbach, Germany). The LC-GC instrument consists of an HPLC equipped with a UV detector which has been coupled to a GC-FID via a loop-type interface. γ-Oryzanol was separated from other rice lipids by liquid chromatography on a silica gel column (Eurospher Si 100-5, 250 × 2 mm I.D., Knauer, Berlin, Germany) using a mixture of hexane/*tert*-butyl methyl ether/2-propanol (95:5:0.5, v/v/v) as the eluent. For detection and quantification of γ-oryzanol, the UV detector was set to 325 nm. The valve of the loop-type interface was switched when the γ-oryzanol-containing fraction was in the loop (560 μL), so that γ-oryzanol was transferred to GC by the carrier gas of the GC system. Transfer occurred by concurrent eluent evaporation at 140 °C. GC separation of the steryl ferulates was performed on a trifluoropropyl-methyl polysiloxane column (Rtx-200MS, 27 m × 0.25 mm i.d. (inside diameter), 0.10 μm film thickness, Restek, Bad Homburg, Germany) connected in series with an uncoated phenylsilylated fused silica capillary (3 m × 0.53 mm i.d.) and a coated precolumn (3 m × 0.25 mm i.d.) having the same coating as the analytical column. During

Table 1. γ-Oryzanol Contents and Compositions of Steryl Ferulates in Brown Rice Samples Obtained from European Cultivars

			steryl ferulates, proportions in total γ-oryzanol (%)				
Cultivar	site (season)	γ-oryzanol content (mg/ 100 g)[a]	campesteryl ferulate	campestanyl ferulate	β-sitosteryl ferulate	cycloartenyl ferulate	24-methylene-cycloartanyl ferulate
			Long grain cultivars				
Thaibonnet	Italy (2000)	32.4 ± 1.1	9.9 ± 0.3	7.5 ± 0.3	8.2 ± 0.3	47.5 ± 0.6	26.9 ± 0.5
Savio	France (2000)	38.5 ± 1.2	6.7 ± 0.2	13.1 ± 0.2	7.1 ± 0.3	44.8 ± 0.5	28.3 ± 0.3
Sigalon	Italy (2000)	36.0 ± 1.2	6.0 ± 0.2	12.4 ± 0.2	7.3 ± 0.1	48.0 ± 0.3	26.3 ± 0.3
Loto	Italy (2001)	50.7 ± 3.8	7.4 ± 0.1	14.2 ± 0.3	7.9 ± 0.3	44.1 ± 0.3	26.4 ± 0.3
Loto	Italy (2002)	48.0 ± 3.3	7.8 ± 0.1	12.6 ± 0.5	7.8 ± 0.4	44.8 ± 1.1	27.1 ± 0.4
Loto	Italy (2002)	51.6 ± 7.7	7.4 ± 0.2	12.0 ± 0.2	7.6 ± 0.2	43.7 ± 0.2	29.4 ± 0.4
Loto	Italy (2002)	54.4 ± 4.4	7.4 ± 0.5	11.9 ± 0.5	7.6 ± 0.5	44.4 ± 0.6	28.7 ± 0.8
			Short grain cultivars				
Helene	France (2000)	26.2 ± 0.9	4.7 ± 0.1	13.1 ± 0.3	6.7 ± 0.1	47.7 ± 0.2	27.8 ± 0.2
Elio	Italy (2001)	46.4 ± 0.9	7.1 ± 0.1	12.6 ± 0.1	6.4 ± 0.1	42.2 ± 0.4	31.7 ± 0.4
Ambra	Italy (2002)	38.7 ± 1.3	6.7 ± 0.7	10.7 ± 0.5	7.0 ± 0.1	44.8 ± 0.9	30.8 ± 0.4
Gladio	Italy (2001)	41.2 ± 1.1	5.8 ± 0.2	12.4 ± 0.3	7.8 ± 0.2	50.6 ± 0.3	23.4 ± 0.2
Gladio	Italy (2002)	40.6 ± 1.5	5.7 ± 0.2	11.6 ± 0.2	7.5 ± 0.2	51.8 ± 0.5	23.3 ± 0.4
Cripto	France (2000)	42.6 ± 1.6	16.4 ± 0.3	5.0 ± 0.2	8.8 ± 0.1	35.0 ± 0.5	34.9 ± 0.4
Cripto	Italy (2000)	45.8 ± 2.8	8.8 ± 0.3	9.8 ± 0.2	6.2 ± 0.2	45.4 ± 0.3	29.8 ± 0.4
Cripto	Italy (2001)	62.7 ± 3.2	7.3 ± 0.2	12.5 ± 0.4	5.4 ± 0.4	47.2 ± 0.7	27.8 ± 0.4
Cripto	Italy (2001)	61.3 ± 2.6	7.0 ± 0.1	12.4 ± 0.1	5.3 ± 0.1	47.8 ± 0.4	27.5 ± 0.4
Cripto	Italy (2002)	51.3 ± 0.8	6.6 ± 0.2	11.2 ± 0.2	6.6 ± 0.3	48.1 ± 0.7	27.5 ± 0.1
Perla	Italy (2000)	43.2 ± 1.1	16.7 ± 0.2	5.0 ± 0.1	8.9 ± 0.2	33.9 ± 0.3	35.5 ± 0.3
Perla	Italy (2001)	42.0 ± 1.9	14.3 ± 0.2	4.2 ± 0.3	8.2 ± 0.3	37.8 ± 0.4	35.5 ± 0.5
Perla	Italy (2002)	37.5 ± 1.6	13.2 ± 0.2	3.4 ± 0.6	9.4 ± 0.1	39.5 ± 0.4	34.5 ± 0.3
Balilla	Spain (2000)	31.8 ± 1.0	14.8 ± 0.2	4.5 ± 0.3	8.5 ± 0.2	35.1 ± 0.5	37.1 ± 0.5
Balilla	Italy (2000)	39.3 ± 0.8	17.7 ± 0.3	5.9 ± 0.1	8.3 ± 0.2	32.4 ± 0.3	35.7 ± 0.5
Balilla	Italy (2002)	39.5 ± 2.7	14.4 ± 0.6	4.1 ± 0.7	9.0 ± 0.2	36.2 ± 0.3	36.3 ± 0.2
Balilla	Italy (2002)	40.3 ± 3.8	13.0 ± 0.6	4.6 ± 0.1	8.6 ± 0.4	36.8 ± 1.0	36.9 ± 0.3
Selenio	France (2000)	34.9 ± 1.1	12.2 ± 0.2	7.0 ± 0.2	9.9 ± 0.2	39.6 ± 0.3	31.3 ± 0.4
Selenio	Italy (2000)	35.3 ± 0.1	13.4 ± 0.8	6.0 ± 0.4	9.1 ± 0.1	37.7 ± 1.4	33.7 ± 0.7
Selenio	Italy (2001)	36.5 ± 1.5	13.9 ± 0.3	4.9 ± 0.2	9.1 ± 0.4	37.1 ± 0.6	35.0 ± 0.3
Selenio	Italy (2002)	43.0 ± 4.2	6.9 ± 0.8	10.5 ± 0.5	7.6 ± 0.2	44.6 ± 0.6	30.4 ± 0.6
Selenio	Italy (2002)	39.7 ± 2.2	13.6 ± 0.7	4.2 ± 0.4	9.6 ± 0.4	39.8 ± 0.7	32.9 ± 0.8
Selenio	Italy (2002)	41.0 ± 5.2	13.4 ± 0.5	3.9 ± 0.2	9.2 ± 0.2	39.5 ± 0.7	34.1 ± 0.2
Mean		41.7	10.2	8.8	7.9	42.3	30.9
Minimum to maximum		26.2–62.7	4.7–17.7	3.4–14.2	5.3–9.9	32.4–51.8	23.3–37.1

[a] In industrially dried material (water content 13%).

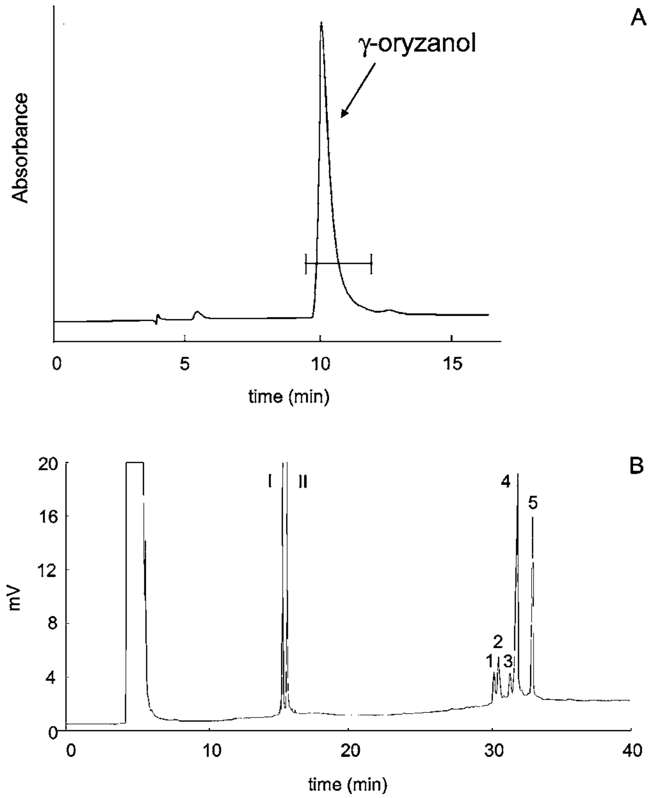

Figure 2. On-line LC-GC analysis of γ-oryzanol in a crude lipid extract from brown rice. (**A**) LC chromatogram; UV detection of γ-oryzanol at 325 nm. The transferred fraction is shown by the indicated time window. (**B**) GC chromatogram of the γ-oryzanol-containing fraction; separation of γ-oryzanol into campesteryl ferulate (1), campestanyl ferulate (2), β-sitosteryl ferulate (3), cycloartenyl ferulate (4), and 24-methylene-cycloartanyl ferulate (5). (I) and (II) were identified as the co-transferred free sterols cycloartenol and 24-methylenecycloartanol.

transfer, solvent vapor was released by an early solvent vapor exit, which was installed between the coated precolumn and the separation column. After transfer, the vapor exit was switched to a restrictor leaving a small purge flow during analysis. Hydrogen was used as the carrier gas with an inlet pressure behind the flow regulator (1.9 mL/min measured at 140 °C) of 250 kPa. After holding the transfer temperature of 140 °C for 5 min, the temperature was programmed to 310 °C at 15°/min and after holding for 5 min to 340 °C at 2.5°/min, which was held for 3 min. The FID temperature was set to 320 °C.

Calibration of the instrument was performed using standard solutions of γ-oryzanol (Henry Lamotte, Bremen, Germany) in hexane (10−40 μg/mL). A calibration curve for determination of γ-oryzanol content was derived by linear regression of the peak area obtained by LC analysis and UV detection at 325 nm. Proportions of individual steryl ferulates were calculated from the peak area ratios obtained by the on-line coupled GC-FID analysis. Calibration and performance of the LC-GC instrument were confirmed daily using a standard solution of γ-oryzanol in hexane (20 μg/mL). Samples of the years 2000 and 2001 were analyzed 6-fold, and samples of the year 2002 were analyzed in triplicate.

Statistical Analysis. Data presented are means ± confidence intervals (error probability 5%). Means were considered as statistically significantly different if their confidence intervals do not overlap.

RESULTS AND DISCUSSION

On-line LC-GC Analysis of γ-Oryzanol. The on-line coupling of a liquid chromatographic preseparation with capillary gas chromatography (on-line LC-GC) is an elegant and efficient approach for the analysis of minor constituents in complex matrices, because it avoids laborious off-line purification steps. An approach based on on-line LC-GC has recently been developed for the rapid analysis of γ-oryzanol content and composition of steryl ferulates in rice (*37*). Briefly, total lipids extracted from brown rice flour are subjected to LC-GC without any prior purification. γ-Oryzanol is preseparated by normal-phase HPLC from other rice lipids (**Figure 2**A) and transferred on-line to GC analysis to separate its major constituents, 24-methylenecycloartanyl ferulate, cycloartenyl ferulate, campesteryl ferulate, β-sitosteryl ferulate, and campestanyl ferulate (**Figure 2B**). Total γ-oryzanol content can be quantified by HPLC-UV detection at 325 nm via external calibration. The proportions of individual steryl ferulates can be calculated from the peak area ratios of the GC-FID chromatogram.

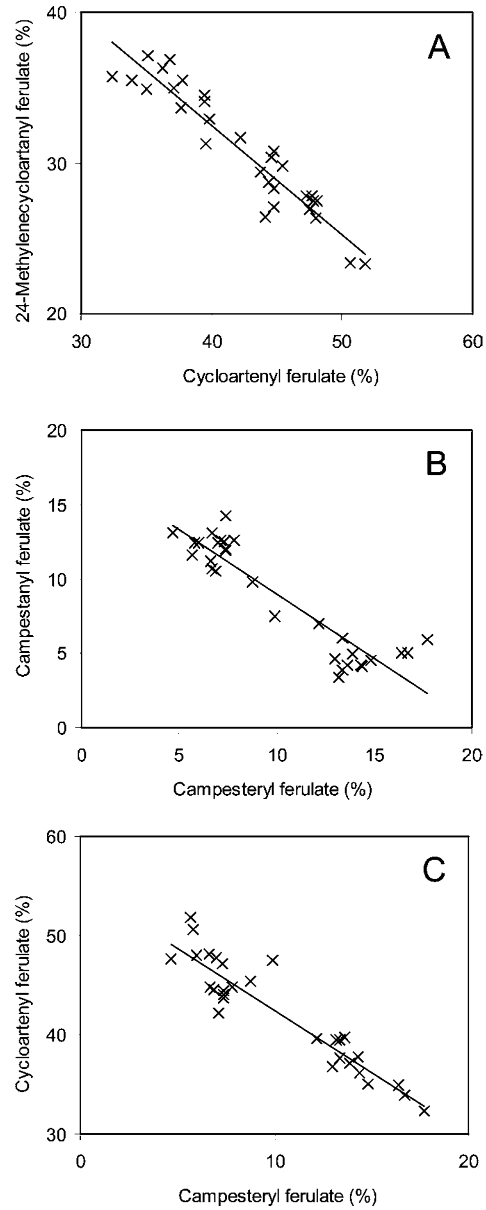

Figure 3. Compositions of steryl ferulates (proportions expressed as the % of total γ-oryzanol) in European brown rice: (**A**) the proportion of 24-methylenecycloartanyl ferulate correlates negatively with the proportion of cycloartenyl ferulate; (**B**) the proportion of campestanyl ferulate negatively correlates with the proportion of campesteryl ferulate; and (**C**) the proportion of cycloartenyl ferulate correlates negatively with the proportion of campesteryl ferulate.

γ-Oryzanol Content in European Brown Rice. To investigate variations in the γ-oryzanol content of European brown rice (industrially dried to a water content of 13%), 30 brown

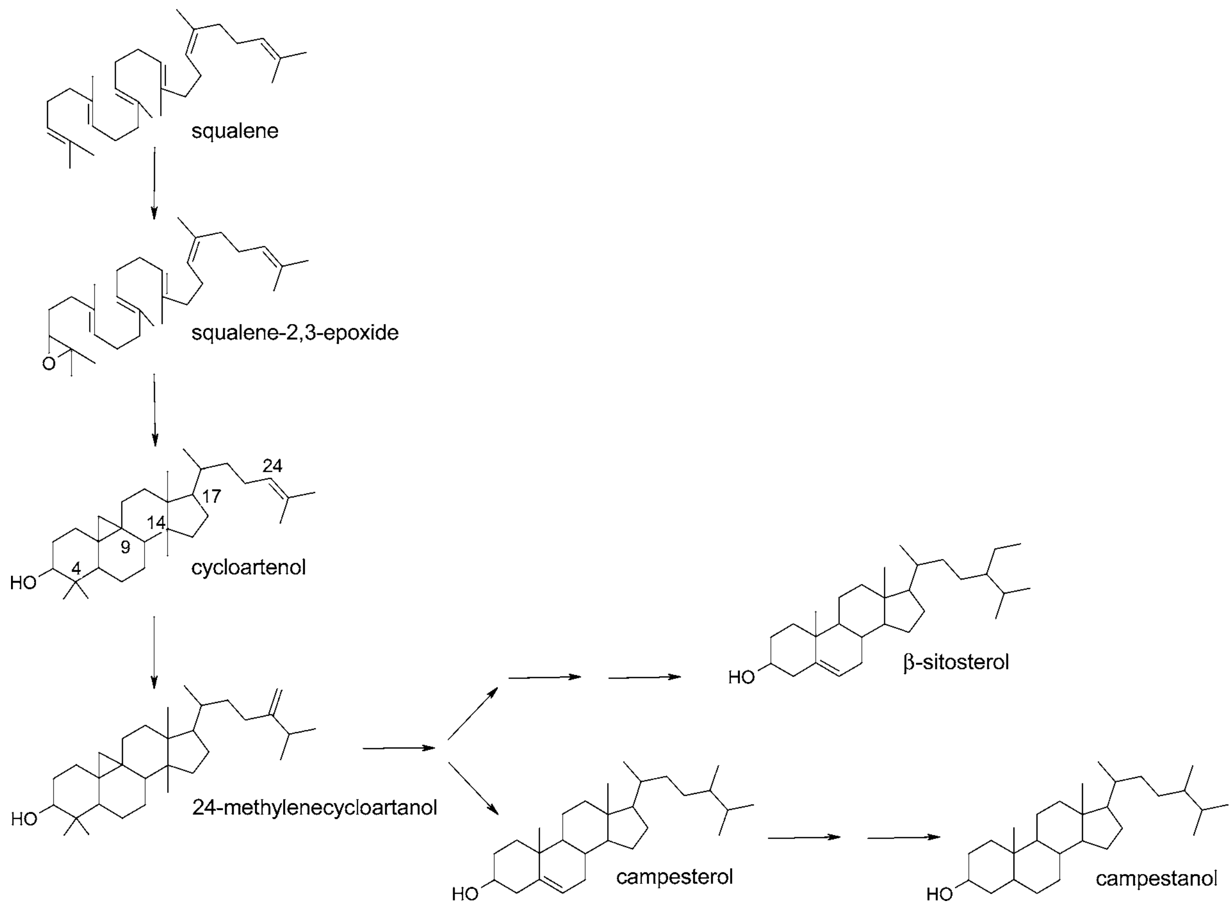

Figure 4. Pathways involved in the biosynthesis of phytosterols.

rice samples obtained from European cultivars grown in Italy, France, and Spain in 2000, 2001, and 2002 were analyzed. Significant variation in γ-oryzanol content was observed (26−63 mg/100 g) (**Table 1**). The γ-oryzanol contents determined in the European samples are of the order of magnitude as reported for individual samples from China (31 mg/100 g), the U.S. (46 mg/100 g), Japan (47 and 48 mg/100 g), and India (62 and 72 mg/100 g) (*7, 34*−*37*). With the upper value being about 2.5 times the lowest value, European brown rice exhibited a range of variation similar to that reported for bran samples of Southern U.S. rice (251−684 mg/100 g) (*22*). Natural variations in steryl ferulate content reported for wheat (6.2−12.3 mg/100 g) and corn (3.1−7.0 mg/100 g) were of similar magnitude (*2*). Irrespective of the significant variation in γ-oryzanol content, all brown rice samples exhibited higher steryl ferulate contents than those reported for wheat (12 mg/100 g), wild rice (9 mg/100 g), corn (7 mg/100 g), rye (6 mg/100 g), triticale (5 mg/100 g), Job's tears (5 mg/100 g), and barley (0.4 mg/100 g) (*2, 4, 7*).

No difference between long and short grain rice was observed for the γ-oryzanol content (**Table 1**). The average γ-oryzanol content in long grain samples (45 mg/ 100 g) was similar to the average γ-oryzanol content in short grain samples (42 mg/100 g).

Analysis of samples obtained from a cultivar grown at different sites or in different seasons revealed that environmental conditions influence γ-oryzanol content (**Table 1**). For example, a sample of cultivar Balilla grown in 2000 in Spain exhibited a statistically significant different γ-oryzanol content (31.8 mg/100 g) compared to a sample grown in Italy in the same season (39.3 mg/100 g). The γ-oryzanol content of a Cripto sample grown in Italy in 2000 (45.8 mg/100 g) was statistically significantly lower than the γ-oryzanol content of a Cripto sample grown in Italy in 2001 (62.7 mg/100 g).

Compositions of Steryl Ferulates in European Brown Rice. Compositions of steryl ferulates in the European brown rice samples are presented in **Table 1**. In all samples, cycloartenyl ferulate and 24-methylenecycloartanyl ferulate were the major constituents of γ-oryzanol. Minor constituents were campesteryl ferulate, campestanyl ferulate, and β-sitosteryl ferulate. This is in accordance with data reported for rice bran and crude rice bran oil (*1, 4, 5, 23*). However, proportions of individual steryl ferulates exhibited enormous variability. The range of variation was much higher than the range of variation reported for the compositions of steryl ferulates in wheat and corn (*2*).

The composition of steryl ferulates was influenced by environmental conditions. For example, in a sample obtained from the cultivar Cripto grown in France in 2000, the proportion of campesteryl ferulate was 3 times the proportion of campestanyl ferulate (**Table 1**). However, a sample of the same cultivar grown in Italy in 2000 exhibited equal proportions of these two steryl ferulates. In a Cripto sample grown in Italy in the following year, the proportion of campesteryl ferulate was half the proportion of campestanyl ferulate. The actual environmental growing conditions for the samples available for analysis were not known. Further studies will be required to elucidate the correlation between factors such as soil quality or climate and the composition of steryl ferulates.

No difference between long and short grain rice was observed for the composition of steryl ferulates. In addition, no correlation between γ-oryzanol content and the composition of steryl ferulates was found.

Surprisingly, irrespective of the great variations observed for proportions of individual steryl ferulates, proportions of the sum

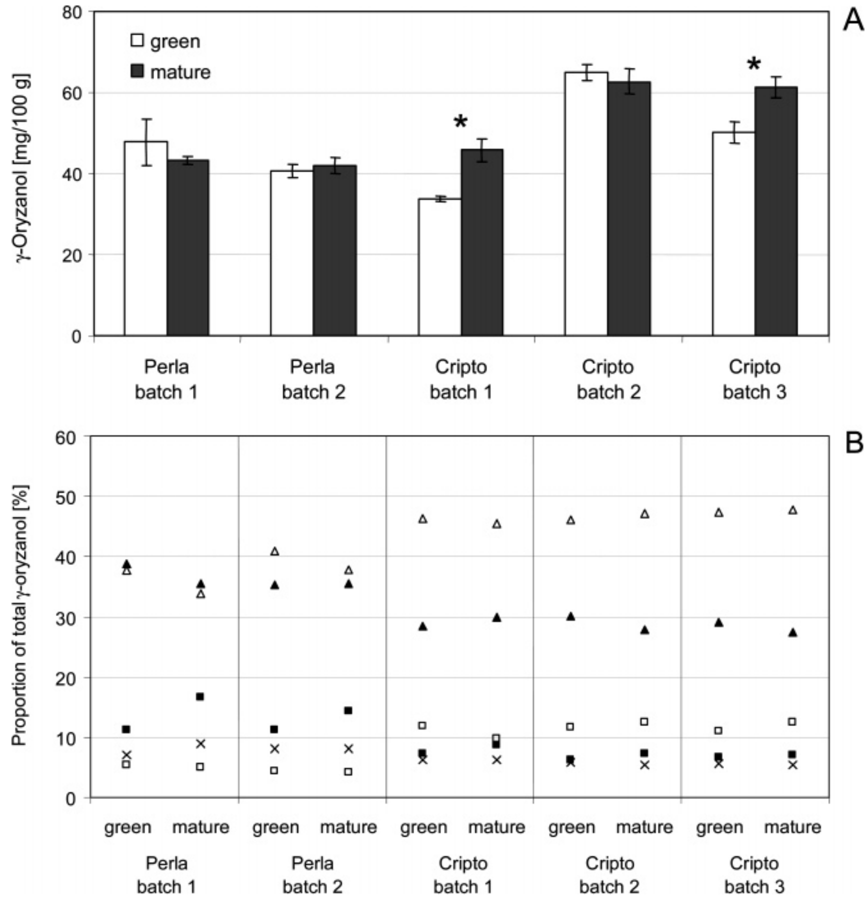

Figure 5. (**A**) γ-Oryzanol content in green and mature rice grains of cultivars Perla and Cripto (industrially dried material; water content 13%). Error bars indicate confidence intervals ($p < 0.05$; $n = 6$). A statistically significant difference between green and mature grains is indicated by an asterisk ($p < 0.05$). (**B**) Compositions of steryl ferulates in green and mature rice grains of cultivars Perla and Cripto are as follows: proportions (% of total γ-oryzanol) for cycloartenyl ferulate (△), 24-methylenecycloartanyl ferulate (▲), campesteryl ferulate (■), campestanyl ferulate (□), and β-sitosteryl ferulate (×).

of 4,4′-dimethylsteryl ferulates (cycloartenyl ferulate, 24-methylenecycloartanyl ferulate) and the sum of 4-desmethylsteryl ferulates (campesteryl ferulate, campestanyl ferulate, and β-sitosteryl ferulate) were rather constant (68−76% and 24−32%, respectively). This was due to the fact that the proportion of cycloartenyl ferulate negatively correlated with the proportion of 24-methylenecycloartanyl ferulate ($r^2 = -0.95$) (**Figure 3**A) and the proportion of campesteryl ferulate negatively correlated with the proportion of campestanyl ferulate ($r^2 = -0.92$) (**Figure 3B**). In addition, there was a relationship between the group of 4,4′-dimethylsteryl ferulates and the group of 4-desmethylsteryl ferulates; the proportion of cycloartenyl ferulate correlated negatively with the proportion of campesteryl ferulate ($r^2 = -0.93$) (**Figure 3C**). Correlations between proportions of individual sterols/steryl derivatives have been reported for olive oil and dandelion leaves; in the unsaponifable matter of virgin olive oil, the proportion of β-sitosterol correlated negatively with the proportion of Δ^5-avenasterol (*38*). Analysis of steryl fatty acid esters in dandelion leaves revealed that the proportion of cycloartenyl esters correlated negatively with the proportion of 24-methylenecycloartanyl esters. Irrespective of the great variations observed for proportions of individual steryl esters the proportion of the sum of cycloartenyl esters and 24-methylenecycloartanyl esters was rather constant between 65−75%. It was demonstrated that the ratio of cycloartenol to 24-methylenecycloartanol is influenced by the season (*39*).

Phytosterols are synthesized from squalene via squalene-2,3-epoxide. After cyclization of squalene-2,3-epoxide, cycloartenol is the first phytosterol produced within the pathway of phytosterols (**Figure 4**). 24-Methylenecycloartanol and campesterol can be considered as intermediates in the biosynthesis of campestanol from cycloartenol (*40*). In the present study, it was observed that the higher the proportions of ferulic acid esters of the two intermediates 24-methylenecycloartanol and campesterol the lower are the proportions of ferulic acid esters of the "early" and "late" phytosterols cycloartenol and campestanol, respectively. Too little is known about the biosynthesis of steryl ferulates to give an explanation for this result. The observed correlations between the proportions of individual steryl ferulates may be due to the ratios of free sterols available for steryl ferulate synthesis. However, the composition of steryl ferulates may be independent from the pool of free sterols but regulated by substrate-specific esterification of the sterols.

Influence of Maturity. Green, immature rice grains are sorted out during industrial rice processing. Rice oil produced from green rice grains has been reported to exhibit a γ-oryzanol content similar to the γ-oryzanol content of rice bran oil produced from mature grains. Therefore, green rice grains have been proposed as material for production of nutraceuticals rich in γ-oryzanol (*41*). However, no information is available as to whether the composition of steryl ferulates in immature grains is the same as that in mature grains.

Three batches of rough rice grains obtained from the cultivar Cripto and two batches obtained from the cultivar Perla were manually dehulled and divided into green and mature grains. This procedure assured that green and corresponding mature grains had the same genetic background and were grown under identical environmental conditions. Due to the industrial drying procedure applied to the rough rice, both rice materials had the same water content (13%). Green and mature grains of each batch were analyzed separately.

Green grains of two batches exhibited statistically significantly lower γ-oryzanol contents compared with their mature counterparts (−18 and −26%); no statistically significant difference between green and mature grains was observed for the remaining three batches (**Figure 5**A). Although some statistically significant differences in steryl ferulate patterns of green and mature grains were detected (**Figure 5B**), differences observed were small compared to the great variations observed for different brown rice samples. Both immature and mature grains of the cultivar Perla exhibited higher proportions of campesteryl ferulate than campestanyl ferulate but similar proportions of cycloartenyl ferulate and 24-methylenecycloartanyl ferulate. Irrespective of their degree of maturity, grains of the cultivar Cripto exhibited higher proportions of cycloartenyl ferulate than 24-methylenecycloartanyl ferulate and higher proportions of campestanyl ferulate than campesteryl ferulate. These results indicate that the degree of maturity does not significantly influence γ-oryzanol content or the composition of steryl ferulates in rice grains. This conclusion must be substantiated by analysis of further material. However, according to the data available, the enormous variations observed for γ-oryzanol content and composition of steryl ferulates in European brown rice cannot be attributed to different degrees of maturity.

This study revealed considerable variations in γ-oryzanol content and composition of steryl ferulates in European brown rice samples. It seems plausible that the correlations shown are of similar relevance for brown rice of other origins. The data demonstrate that claims related to cholesterol-lowering and antioxidative effects of rice products have to take into account the compositions of the selected raw materials.

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